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Development of Nerve Cells in Hydrozoan Planulae: III. Some Interstitial Cells Traverse the Ganglionic Pathway in the Endoderm

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Abstract. Hydrozoan planulae of *Pennaria tiarella* possess migratory stem cells—interstitial cells—that are capable of self renewal and can differentiate into either ganglionic nerve cells or nematocytes. The commitment and differentiation of a subpopulation of larval endodermal interstitial cells to the neural pathway were examined using light immunocytochemistry and transmission electron microscopy. Embryos of different ages, from 8 to 96 h, were tested for their ability to bind rabbit antiserum raised to the neuropeptide FMRFamide. A subpopulation of interstitial cells in the anterior endoderm of the planula begins to express a FMRFamide-like antigen between 48 and 72 h postfertilization. Concurrent with this endodermal interstitial cell expression, a subset of ectodermal ganglionic cells with FMRFamide-like immunoreactivity appears in the anterior end of the planula. Ultrastructural examination of the interstitial cell population in the anterior planular endoderm, at 48 h in development, indicates that, based upon morphology, there are at least three subsets of interstitial cells in this region: undifferentiated interstitial cells, interstitial cells traversing the nematocyte differentiation pathway, and interstitial cells traversing the neural differentiation pathway. The endodermal interstitial cells entering the neural pathway form a Golgi complex, electron-dense droplets, dense cored vesicles, and microtubules. Neurite formation does not occur in the endoderm; rather, neurites are only found in association with ectodermal ganglionic cells. Furthermore, planulae lack fully differentiated endodermal neurons. This study demonstrates that, during embryogenesis, some interstitial cells destined for neural differentiation are committed in the endoderm before their emigration to the ectoderm, begin to express cytochemical and morphological features of neural differentiation while in the endoderm, and migrate to the ectoderm as neuroblasts.

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Introduction

The hydrozoan planula larva is an especially good system with which to examine the commitment and differentiation of cells during development: the number of cell types in the larva is small; their arrangement is simple; and neither the variety nor the arrangement of larval cells are very far from those of the adult (Martin and Thomas, 1980; Martin *et al.*, 1983; Thomas *et al.*, 1987; Martin, 1988a, b, c).

The hydrozoan planula contains a population of migratory undifferentiated cells: interstitial cells. Interstitial cells are capable of self-renewal, and can differentiate into either ganglionic nerve cells or nematocytes (Martin and Thomas, 1981a, b; Martin, 1988a). Interstitial cells arise in the endoderm, later migrate into and populate the ectoderm, and eventually differentiate into the two classes of cells (Martin and Archer, 1986). Do the interstitial cells (1) migrate as uncommitted cells and become committed by some sort of positional cues upon arrival in the ectoderm, or (2) are committed before they leave the endoderm, and migrate into the ectoderm to complete differentiation? The second alternative is correct for nematocytes, (Martin and Archer, 1986), and in this paper I show that it is also correct for neurons (*i.e.*, ganglionic cells).

This research describes a series of histological experiments designed to determine whether interstitial cells in a hydrozoan planula develop neuronal characteristics (ganglionic features) before arriving at their final destination in the ectoderm. The numbers and locations of in-

terstitial cells and ganglionic cells in hydrozoan embryos of different ages were determined by light microscopy and transmission electron microscopy (TEM). The ability of these embryos to bind a rabbit antiserum raised to the neuropeptide FMRFamide [such immunoreactivity has been demonstrated in planular sensory cells (Martin, 1988b)] was tested to determine whether the antigen is expressed by ganglionic cells or interstitial cells differentiating along the ganglionic pathway. Anti-FMRFamide was used in this study because when it is applied to cnidarians, the peptides bound are likely to be related to pGlu-Gly-Arg-Phe-amide, which is present in large amounts in nervous systems of adult anthozoans and probably also in hydrozoans and scyphozoans (Graff and Grimmelikhuijzen, 1988). The planular results show that a subpopulation of interstitial cells in the anterior endoderm of 48 h planulae expresses morphological and cytochemical features of ganglionic cell differentiation. Thus, at least some interstitial cells for the neural differentiation pathway are committed in the endoderm and actually traverse the ganglionic pathway in the endoderm.

Materials and Methods

Mature colonies of *Pennaria tiarella* were collected from pier pilings in Morehead City, North Carolina. Fronds from male and female colonies were placed together in the dark at 6:00 pm. At 9:00 pm the bowls were returned to the light and, within an hour, early cleavage embryos were found in the bottoms of the dishes. Embryos were collected, placed in small finger bowls of filtered seawater, and reared at 23°C.

Embryos of seven different ages: 8-, 10-, 16-, 24-, 48-, 72-, and 96-h, were prepared for transmission electron microscopy. Animals were fixed for 1 h in 2.5% glutaraldehyde, pH 7.4, in 0.2 *M* phosphate buffer. They were subsequently postfixed for 1 h in 2% osmium tetroxide (pH 7.2, in 1.25% sodium bicarbonate), dehydrated in an ethanol series, infiltrated, and embedded in Spurr's embedding medium. Serial thick and thin sections were cut with a Porter-Blum MT-2B ultramicrotome. Thick sections were mounted on gelatin-coated slides, stained with 0.5% toluidine blue in 1% sodium borate, and examined with a Zeiss research microscope. Thin sections were placed on 150-mesh copper grids and stained with 3.5% uranyl acetate in ethanol followed by lead hydroxide. Grids were examined and photographed with a Hitachi H-600 transmission electron microscope.

Wholemounts and paraffin sections of the selected embryonic stages were tested for their ability to bind a rabbit antiserum raised to FMRFamide (Immuno Nuclear Corporation). Twenty-four-hour planulae, treated for 2 h with 0.2% colchicine in seawater and subsequently allowed to recover for 24 h, were also exposed to the FMRFamide antiserum. Such colchicine treatment eliminates the entire interstitial cell system *i.e.*, interstitial cells, nematoblasts, nematocytes, and ganglionic cells (Martin and Thomas, 1981b).

To visualize the binding of FMRFamide antiserum on wholemounts, the procedure presented by Martin (1988b) was followed with some modifications. Animals were fixed for 1 h in 10% formalin in seawater and subsequently washed 3 times, for 15 min each, in 10 m*M* phosphate-buffered saline (PBS, pH 7.2). Incubation with the FMRFamide antiserum was for 1–4 h, with the primary antibody diluted 1:200 with 10 m*M* PBS, pH 7.2, containing 0.1% sodium azide, 0.3% Triton X-100, and 2% fetal calf serum. Incubations were carried out with planulae in lid-covered 96 well tissue culture plates placed on a rotating shaker platform set at 60 rpm. At the end of the first incubation period, the primary antibody was removed, and animals were washed 3 times, for 15 min each, in 10 m*M* PBS, pH 7.2. Incubation with the second antibody was for 1 h in fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobin (Boehringer Mannheim). The FITC-tagged antibody was diluted 1:120 in 10 m*M* PBS, pH 7.2, containing 0.1% sodium azide, 0.3% Triton X-100, and 10% fetal calf serum. The second incubations were also done in 96 well plates rotated at 60 rpm. After the second incubation, animals were washed for three 15-minute changes in 10 m*M* PBS, pH 7.2. Wholemounts were examined for fluorescently labeled cells with a Zeiss microscope equipped with epifluorescence.

To visualize binding of FMRFamide antiserum to paraffin sections of embryos, samples fixed in formalin were dehydrated through an alcohol series, infiltrated and embedded in paraffin, and serially sectioned at 8 μm. Nine sections were mounted in the center of a glass slide, three rows one above the other, and each row containing three sections. Slides were rehydrated to distilled water, and the sections were surrounded by an outer ring of vacuum grease. Grease application was done in a moist chamber to prevent the sections from drying. FMRFamide antiserum was placed in the grease-created wells, thus immersing the sections. The slides were placed in a covered moist chamber and rotated at 20–40 rpm for 1–4 h. PBS rinses and incubation in the second antibody were carried out in the moist chamber. After incubation, the grease was removed from the slides; the sections were covered with mineral oil and examined for fluorescently labeled cells.

Binding specificity of the FMRFamide antiserum was determined by preincubating a 1:200 dilution of the antiserum with 10 μg/ml synthetic FMRFamide (Peninsula Lab) for 24 h at 4°C before using it to stain the embryos.

Results

Mature planula (72–96 h postfertilization)

The mature planula consists of an ectoderm, an acellular mesoglea, and an endoderm (Fig. 1). The ectoderm contains epithelial cells (epitheliomuscular, glandular, and sensory), interstitial cells, and their derivatives (nematoblasts, nematocytes, and ganglionic cells), whereas, the endoderm has gastrodermal epithelial cells, interstitial cells, and nematoblasts. Interstitial cells, nematoblasts, nematocytes, and ganglionic cells are easily identified in planular tissue at the light microscopic level (Figs. 1–3). Interstitial cells—small round cells measuring 7.5 μm in diameter—contain a centrally located nucleus with one to several nucleoli. They possess few cytoplasmic organelles and are scattered among the epithelial cells in both the ectoderm and the endoderm along the entire anterior-posterior axis of the planula (Fig. 2). Nematoblasts (developing nematocytes) range from 10 to 12.5 μm in diameter and have distinctive dark- or light-staining capsules (Figs. 1, 2). Each capsule houses a nematocyst thread that may possess barbs and spines. Nematoblasts are located in both the ectoderm and the endoderm and are mostly confined to the anterior and middle two-thirds of the planular axis. Mature nematocytes are found only at the ectodermal surfaces of planulae and exhibit the same distribution pattern as that of the nematoblasts. Ganglionic cells are 5 μm in diameter, exhibit a spindle shape, and are positioned all along the planular anterior-posterior axis at the base of the ectoderm just above the mesoglea (Fig. 3). The ganglionic perikaryon, its long axis oriented parallel to the mesoglea, contains a Golgi complex, microtubules, mitochondria, electron-dense droplets, and dense cored vesicles. Neurites project from either side of the cell bodies and form an extensive ectodermal neural plexus above the mesoglea (Figs. 3, 4). Such neurites are filled with microtubules, mitochondria, electron-dense droplets, and dense cored vesicles (Fig. 4). Electron-dense droplets and dense cored vesicles are found exclusively in differentiating and full-differentiated nerves. The endoderm lacks ganglionic cells and a neural plexus.

Interstitial cells and nematoblasts are migratory, whereas, nematocytes and ganglionic cells are not (Martin and Archer, 1986). These migratory cells move as single cells, and migration has been observed from the endoderm to the ectoderm and, once in the ectoderm, up and down the planular axis.

FMRFamide-like immunoreactivity is detected in a subpopulation of ganglionic cells in the planular ectoderm at 72 h postfertilization, just before metamorphosis (Fig. 5). Such immunopositive nerve cells are located at the base of the ectoderm above the mesoglea and are confined to the anterior head and anterior sides of the plan-

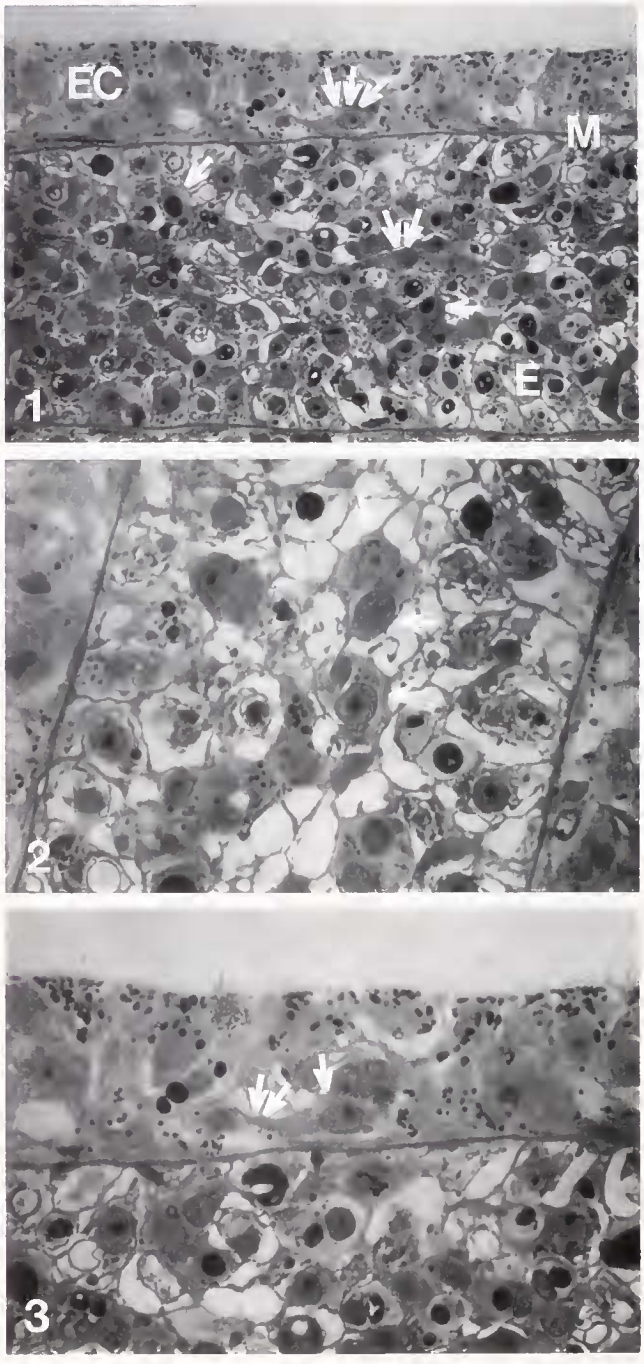


Figure 1. Longitudinal section of a 72 h planula showing ectoderm (EC), mesoglea (M) and endoderm (E). Endodermal nematoblasts (single arrow) and interstitial cells (double arrows) and an ectodermal ganglionic cell (triple arrows) are visible. ×250.

Figure 2. Endodermal interstitial cells (arrows) in a 72 h planula. Each cell contains a large nucleus with a prominent nucleolus and few other cytoplasmic organelles. ×620.

Figure 3. Ectodermal ganglionic cell (arrow) in a 72 h planula. The cell body is oriented parallel to the mesoglea and neurites (double arrows) extend from each side of the perikaryon. ×620.

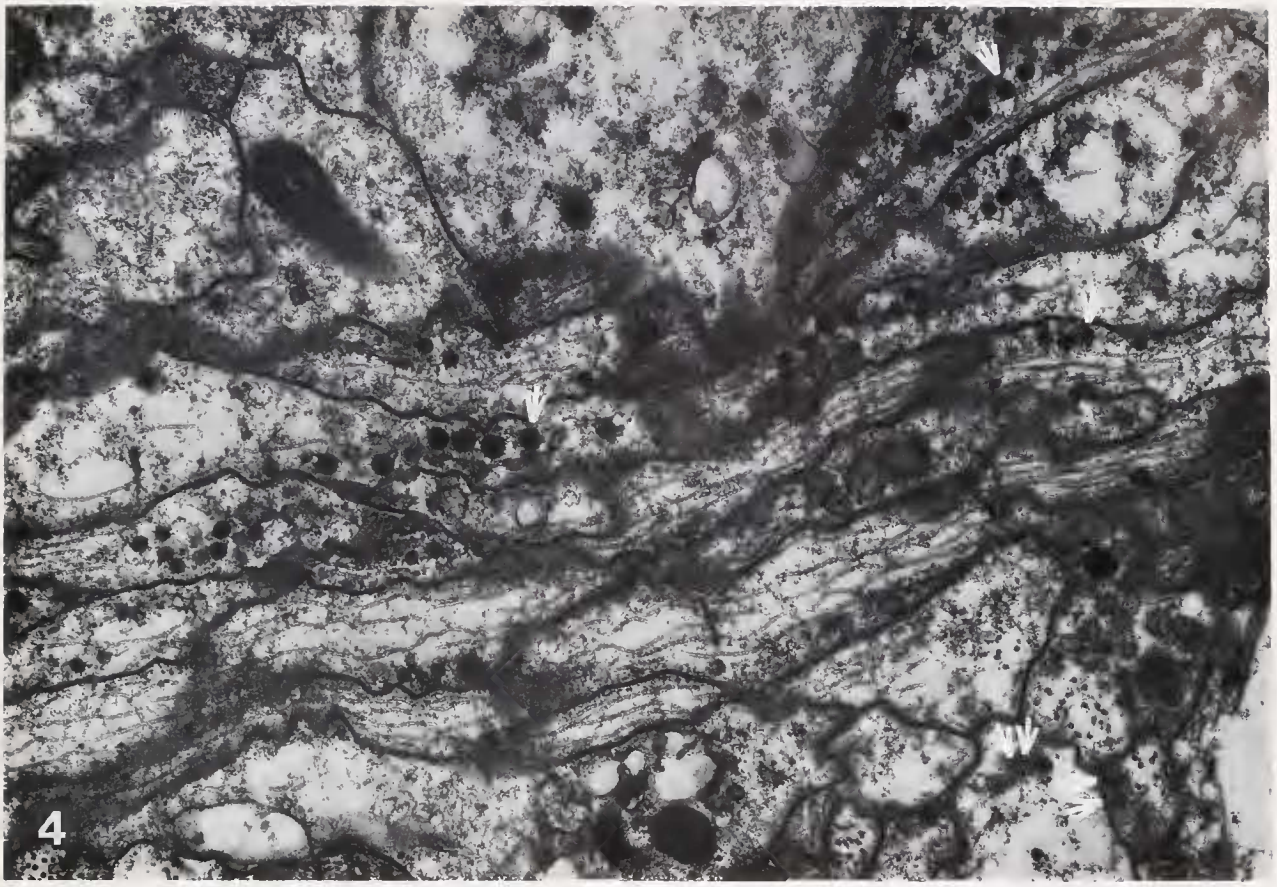


Figure 4. Ectodermal neurites of ganglionic cells. These neurites form a plexus just above the mesoglea and are rich in microtubules, mitochondria, electron-dense droplets (single arrows) and dense cored vesicles (double arrows). ×19,000.

ula. Cell bodies of the immunopositive ganglionic cells are stained, whereas their neurites (processes) are not. Nematoblasts and nematocytes do not produce the FMRFamide-like peptide, as indicated by their lack of staining. Furthermore, the majority of planular interstitial cells do not stain with the antibody. There is, however, a small subset of interstitial cells in the anterior endoderm of 72-h planulae that does express a FMRFamide-like peptide. Such positive cells first produce the neuropeptide at 48 h in development and are described below (see *Forty-eight hour planula*).

Figure 5. Wholemount of a 72 h planula. A subpopulation of ganglionic cells (arrow) in the ectoderm of the planula exhibits FMRFamide-like immunoreactivity. Such ectodermal ganglionic cells are confined to the anterior head and anterior sides of the planula. ×200.

Gastrulating embryo (8–10 h postfertilization)

Embryos gastrulate between 8–10 h postfertilization resulting in the formation of an immature, 10-h planula. This young planula consists of an ectoderm, an acellular mesoglea, and an endoderm (Figs. 6, 7). The ectoderm contains epithelial cells (dark-staining epitheliomuscular cells and light-staining glandular cells) and is devoid of interstitial cells, nematoblasts, nematocytes, and ganglionic cells. The endoderm consists of an outer epithelial layer of gastrodermal cells surrounding a central core of tightly packed interstitial cells (Figs. 6, 7). This core of interstitial cells extends the entire length of the planular

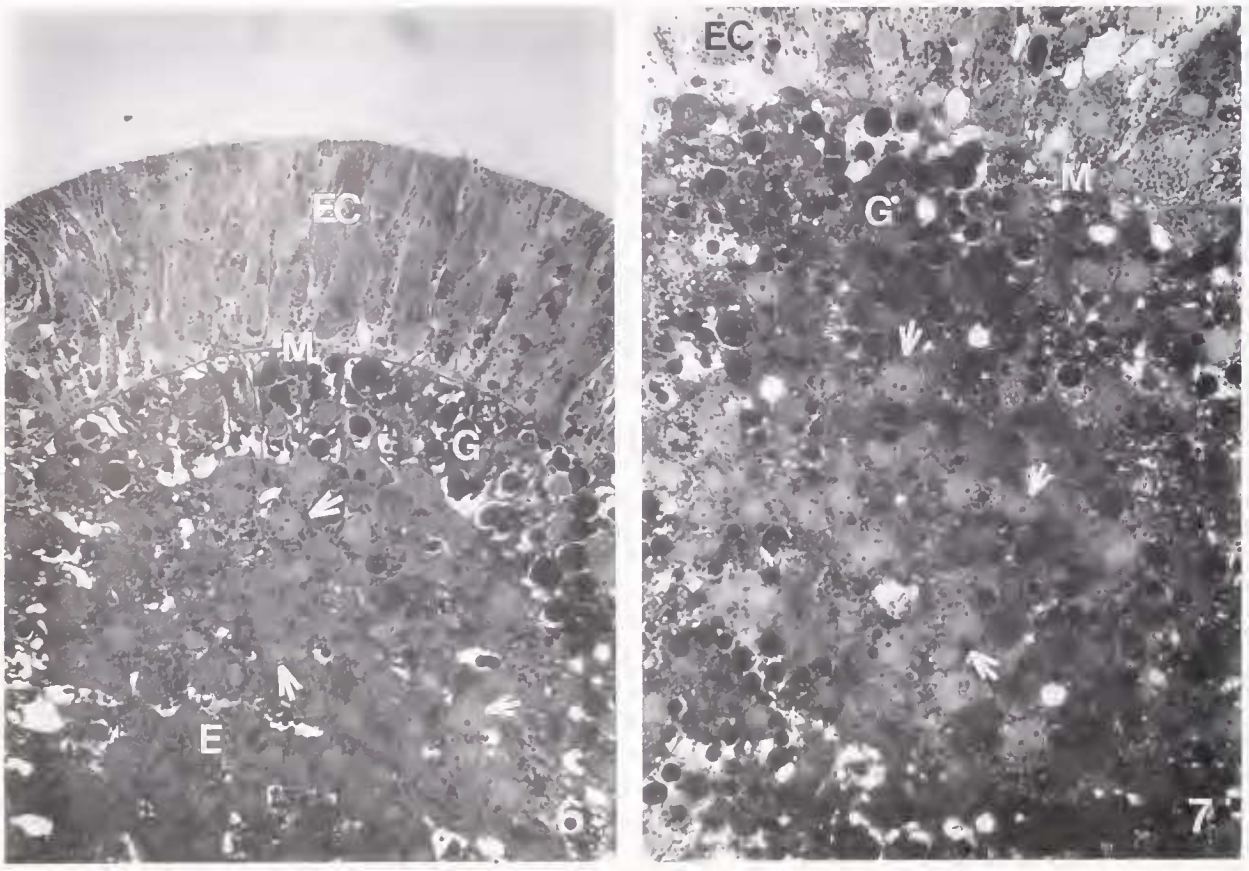


Figure 6. Cross section of a 10-h planula. The embryo consists of an ectoderm (EC), an acellular mesoglea (M), and an endoderm (E). The endoderm is composed of an outer columnar epithelial layer (G) surrounding a central core of lightly staining interstitial cells (arrows). The ectoderm contains epitheliomuscular cells (dark-staining cells) and glandular cells (light-staining cells), but is devoid of interstitial cells, nematoblasts, nematocytes, and ganglionic cells. ×320.

Figure 7. Endodermal region of a 10-h planula. Clusters of interstitial cells (arrows) occupy the central endoderm. EC, ectoderm; G, epithelial layer of endoderm; M, mesoglea. × 320.

anterior-posterior axis. These lightly staining, oval-shaped interstitial cells possess a large, centrally located nucleus with one to several nucleoli. Dark-staining granules occupy the cytoplasm of these young interstitial cells, however, such granules disappear as the cells mature. Interstitial cells of late planulae possess few granules (see Fig. 2).

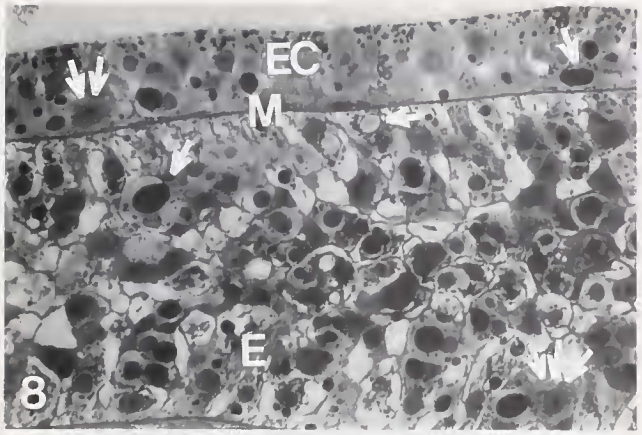
Interstitial cells traverse the nematocyte differentiation pathway in the endoderm (see Figs. 1, 2, 8). Such cells are distinguished by the appearance of either a dark- or light-staining nematocyst capsule. The capsule enlarges to an extent that it displaces the nucleus to one side of the cell. A few endodermal nematoblasts, confined to the anterior and middle two-thirds of the endoderm, have been observed in the 10-h planula. Interstitial cells traversing the neural differentiation pathway have not been seen in the immature planula.

Interstitial cells and nematoblasts emigrate as single cells from the endoderm to the ectoderm. Interstitial cells migrate out from all locations along the planular endodermal axis, whereas outward nematoblast migration is confined to the anterior and middle endodermal regions. Interstitial cells and nematoblasts first appear in the planular ectoderm at 14 h postfertilization (Martin and Archer, 1986). Their ectodermal distribution corresponds to their above-mentioned migration patterns.

Immature planulae (10 h) do not express a FMRF-amide-like antigen as indicated by their lack of staining.

Young planula (24 h postfertilization)

By 24 h, the planular ectoderm contains epithelial cells (epitheliomuscular, glandular, and sensory), interstitial cells, nematoblasts, a few nematocytes, and ganglionic cells; the endoderm has gastrodermal epithelial cells, interstitial cells, and nematoblasts. Both interstitial cells



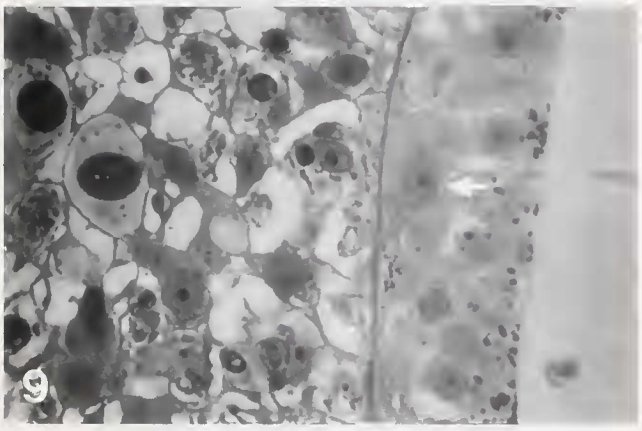


Figure 8. Longitudinal section of a 24-h planula. Differentiating nematoblasts (single arrows) are visible in both the endoderm (E) and the ectoderm (EC). A young ganglionic cell (double arrows) is seen at the base of the ectoderm above the mesoglea (M). Its neurites are not yet fully formed. Triple arrows, endodermal interstitial cells. ×250.

Figure 9. Ectodermal ganglionic cell (arrow) in a 24 h planula. Note its spindle shape and extending neurites. ×620.

and ganglionic cells occupy the entire anterior-posterior axis of the planula, whereas, nematoblasts and nematocytes are confined to the anterior and middle regions of the animal (Figs. 8, 9). In the ectoderm, ganglionic cells and nematoblasts are positioned in close proximity to the mesoglea, and interstitial cells are located slightly above these cells (*i.e.*, toward the outer ectodermal surface). In the endoderm, interstitial cells and nematoblasts may be found in the central core or out closer to the mesoglea. As planulae mature (24–96 h) the numbers of ectodermal and endodermal interstitial cells, ectodermal and endodermal nematoblasts, ectodermal nematocytes, and ectodermal ganglionic cells increase.

At 24 h, the nervous system begins to form (Martin, 1988a, b). This neural system is entirely ectodermal and consists of ganglionic cells (interstitial cell derivatives) and sensory cells (epithelial derivatives). Ganglionic cells form a neural plexus composed of cell bodies and their neurites; this plexus extends the entire length of the planula and is located just above the mesoglea (Fig. 9). These ganglionic cells have originated from interstitial cells that have migrated from the endoderm to the base of the ectoderm. Once in this ectodermal position, they elaborated morphological features characteristic of ganglionic cell differentiation. Interstitial cells traversing the ganglionic pathway in the endoderm have not been observed at this stage. Sensory cells first arise in the anterior end of the planula (later in development they appear all along the length of the planula) and extend from the free surface of the planula to the ganglionic plexus where they insert neurites into the plexus (Martin, 1988b).

Twenty-four hour ganglionic cells do not produce a FMRFamide-like peptide, however, the sensory cells do (Martin, 1988b). The FMRFamide-like peptide is first observed in the apices of sensory cells and only later in their mid to basal regions. FMRFamide-like positive sensory cells are observed throughout the remaining larval period (Figs. 10, 11). Interstitial cells and nematoblasts lack immunostaining at this stage, as does the entire endoderm.

Forty-eight hour planula

The distribution of interstitial cells and their progeny in the 48-h planula is similar to that observed in the 24-h planula. By 48 h, the numbers of these cells have dramatically increased in both germ layers.

At 48 h, a subpopulation of interstitial cells in the anterior endoderm begins to express a FMRFamide-like antigen (Figs. 10, 11). These positive-staining interstitial cells are found exclusively in the anterior-most endodermal region of the planula and are present in the central endodermal core and in the outer endodermal periphery (Figs. 10, 11). Depending upon the plane of section, the FMRFamide-positive interstitial cells exhibit either a mesenchymal shape or an oval morphology. Interstitial cells located in the mid to posterior endodermal regions do not express the FMRFamide-like peptide, as indicated by an absence of staining (Fig. 12). Just after the endodermal appearance of these FMRFamide-like positive interstitial cells, a few immunopositive ganglionic cells are detected in the ectoderm above the mesoglea confined to the anterior head and anterior sides of the planula. Their cell bodies are stained whereas their neurites are not. This is a full day after ganglionic cells first appear in the planular ectoderm. Nematoblasts and nematocytes do not stain for the FMRFamide-like peptide at this stage.

Between 48 and 72 h, the number of immunopositive endodermal interstitial cells and immunopositive ectodermal ganglionic cells increase. Their distribution is limited to the anterior end of the planula.

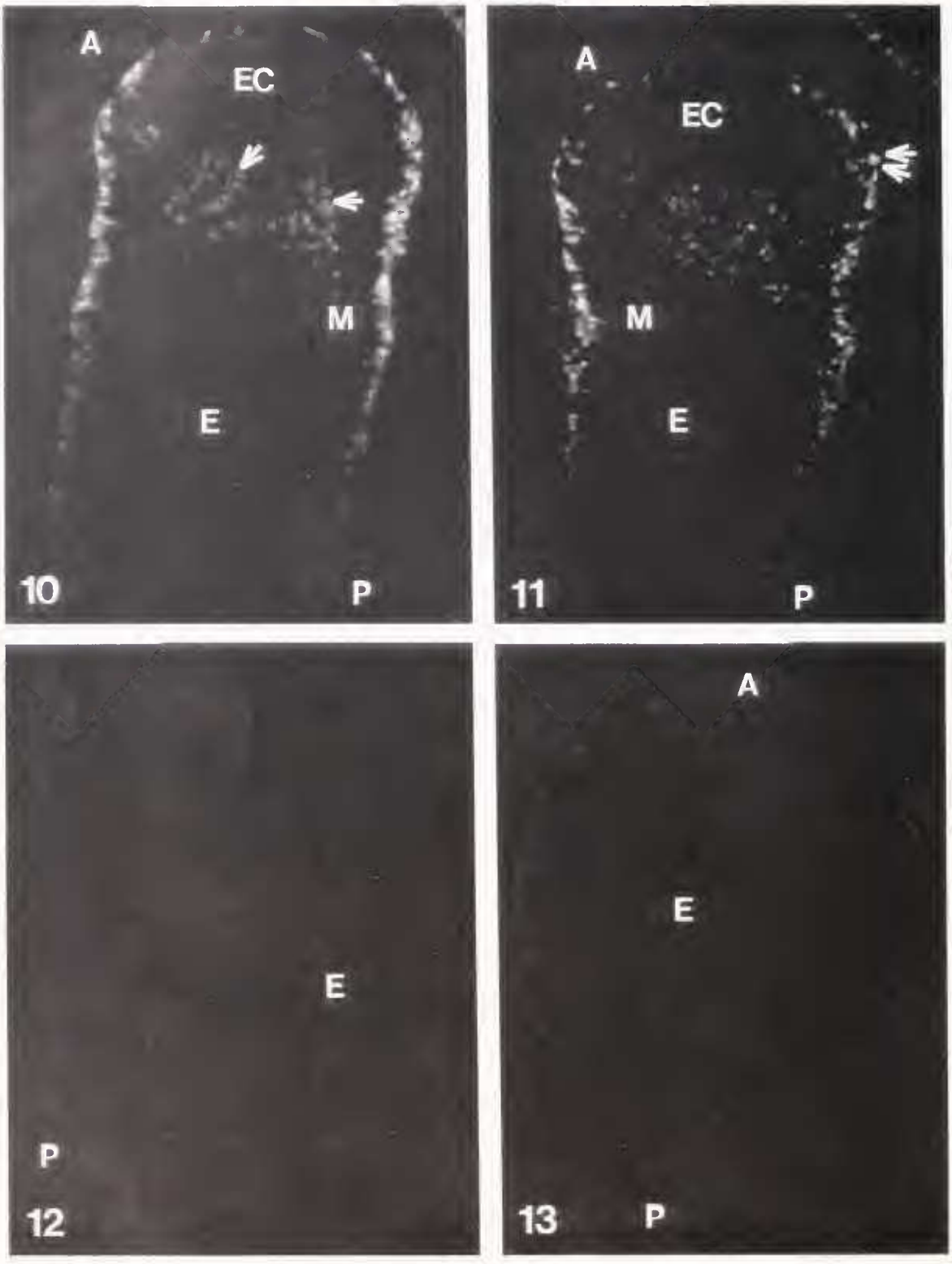


Figure 10. Longitudinal paraffin section of a 48-h planula. A subpopulation of interstitial cells (arrows) in the anterior endoderm of the planula begins to express a FMRFamide-like antigen at this stage in development. A, anterior; E, endoderm; EC, ectoderm; M, mesoglea; P, posterior. ×250.

Figure 11. Longitudinal paraffin section of a 48-h planula. This section is taken from a deeper region of the same planula shown in Figure 10. A subset of interstitial cells in the anterior endoderm expressing a FMRFamide-like antigen is visible, as are FMRFamide-positive sensory cells (double arrows). A, anterior; E, endoderm; EC, ectoderm; M, mesoglea; P, posterior. ×250.

Ultrastructural examination of interstitial cells in the anterior endoderm of 48-h planulae indicates that, based upon morphology, at least three subsets of interstitial cells are found in this region: undifferentiated interstitial cells, interstitial cells traversing the nematocyte differentiation pathway (nematoblasts), and interstitial cells traversing the ganglionic differentiation pathway (neuroblasts) (Figs. 14–19). All three subpopulations can be found in the central endodermal core and at the periphery of the endoderm. These three subsets are also found in older planulae (72 h) in the same endodermal position. Undifferentiated interstitial cells are characterized by a centrally located nucleus with a nucleolus, and a cytoplasm containing free ribosomes, a few mitochondria, and a few segments of rough endoplasmic reticulum (Fig. 14). These interstitial cells, as of yet, show no specific organelles indicative of a particular differentiation pathway. Interstitial cells committed to the nematocyte differentiation pathway have a cytoplasm rich in rough endoplasmic reticulum and form a distinctive nematocyst capsule (Fig. 15). This capsule is in close proximity to the nucleus and often displaces it to one side of the cell. Interstitial cells undergoing neural differentiation (ganglionic cell pathway) form a Golgi complex, electron-dense droplets, dense cored vesicles, and microtubules (Figs. 16–19). These electron-dense droplets and dense cored vesicles occupy the cell bodies of the developing endodermal ganglionic cells and are morphologically identical to the droplets and vesicles found in the ectodermal ganglionic cell bodies and neurites (see Figs. 4, 16, 17, 18, and 19). These developing endodermal neuroblasts do not form neurites in the endoderm, as neurites have only been observed in the ectoderm of the planula.

Colchicine-treated embryos

Embryos treated with colchicine and subsequently allowed to recover for one to two days lack all interstitial cells and their differentiated progeny (ganglionic cells, nematoblasts, neuroblasts, and nematocytes) (Martin and Thomas, 1981a). When such epithelial planulae are exposed to FMRFamide antiserum, they show no immunostaining (Fig. 13). There are no immunopositive interstitial cells, immunopositive neuroblasts, or immunopositive ganglionic cells.

Discussion

Research presented here, as well as past work (Martin and Archer, 1986), indicates that at least some larval interstitial cells are committed within the endoderm to the differentiation of either nerve cells or nematocytes. These restricted cells enter a differentiation pathway in the endoderm, and most probably migrate as nematoblasts or neuroblasts to a position in the ectoderm where differentiation is completed. This process probably accounts for the spatial distribution of the interstitial cell system in the larval ectoderm.

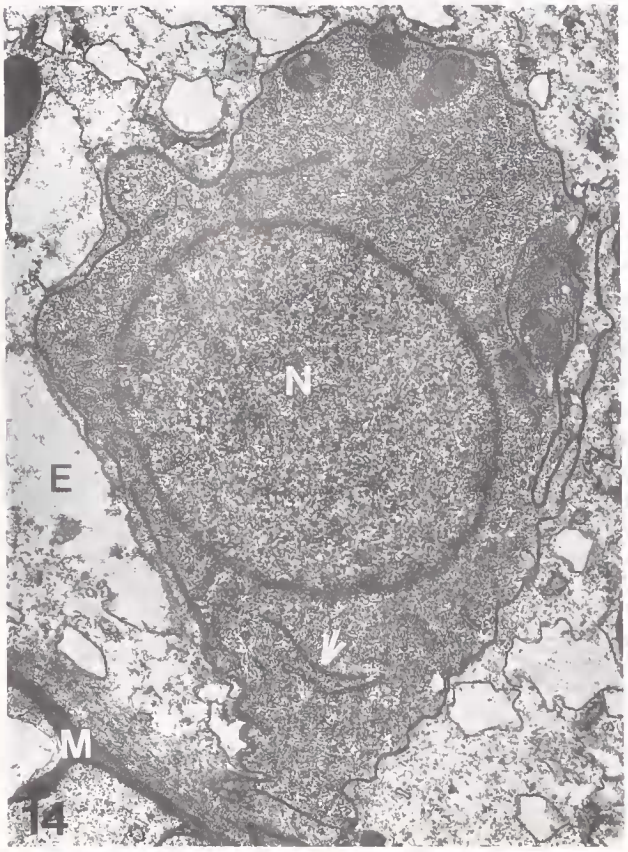
With regard to ganglionic cell formation, this study demonstrates a subpopulation of anterior endodermal interstitial cells that shows early signs of neural cytochemical differentiation by expressing a FMRFamide-like antigen. Concurrent with the appearance of these immunopositive interstitial cells, TEM indicates that a subset of interstitial cells in the same anterior endodermal region develops morphological features indicative of neural differentiation: formation of a Golgi complex, electron-dense droplets, dense cored vesicles, and microtubules. This subset of interstitial cells probably includes the FMRFamide-positive interstitial cells.

Furthermore, a subset of FMRFamide-positive ganglionic cells appears in the anterior ectoderm between 48–72 h. Because this occurs just after the endodermal appearance of the immunopositive interstitial cells, and because both populations are confined to the same anterior head region, the immunopositive interstitial cells have probably migrated to the base of the ectoderm where they differentiated into ganglionic cells. Alternatively, the interstitial cells traversing the neural pathway in the endoderm might never migrate to the ectoderm but simply remain and complete their differentiation, or die, in the endoderm. The alternative is unlikely because the planular endoderm lacks fully differentiated ganglionic cells [*i.e.*, they do not form neurites in the endoderm; neurite formation constitutes the last step in ganglionic cell differentiation (Martin, 1988a)], and TEM studies reveal no signs of degenerating cells in the endoderm at any stage of planular development.

The movements of interstitial cells, nematoblasts, and neuroblasts in planulae appear to be coordinated, as evidenced by their final placement within the ectoderm. Interstitial cells, which divide and possibly remain as stem cells, migrate out from all regions of the endoderm and distribute themselves along the whole planular axis in the ectoderm. Developing nematoblasts emigrate from the endoderm in a specific region of the planula (anterior to mid endoderm) and concentrate in an ectodermal area extending from the anterior end of the planula to the

Figure 12. Paraffin section of the posterior (P) region of a 48 h planula. The posterior endoderm (E) is devoid of FMRFamide-positive interstitial cells. ×250.

Figure 13. Paraffin section of a mature "recovered" colchicine-treated planula. Such epithelial planulae lack FMRFamide-like activity as indicated by the absence of staining. ×250.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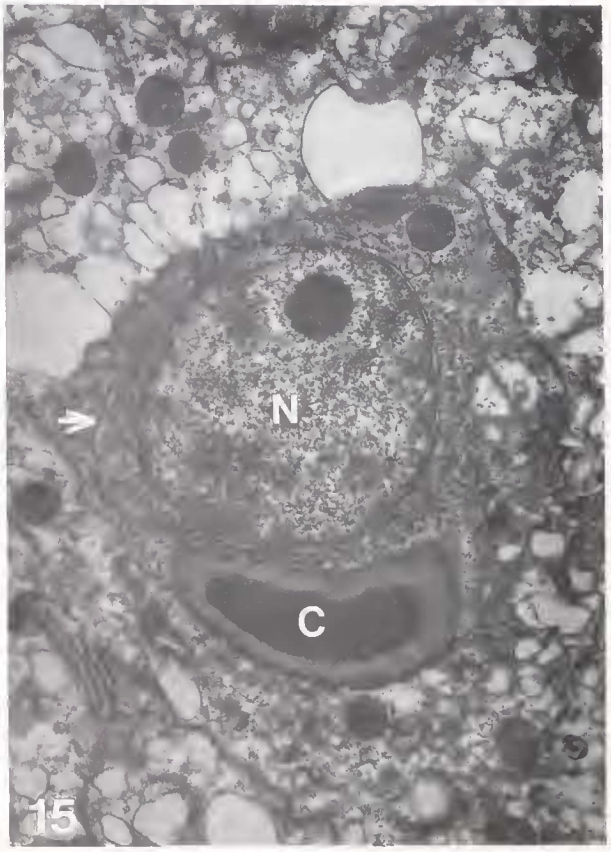
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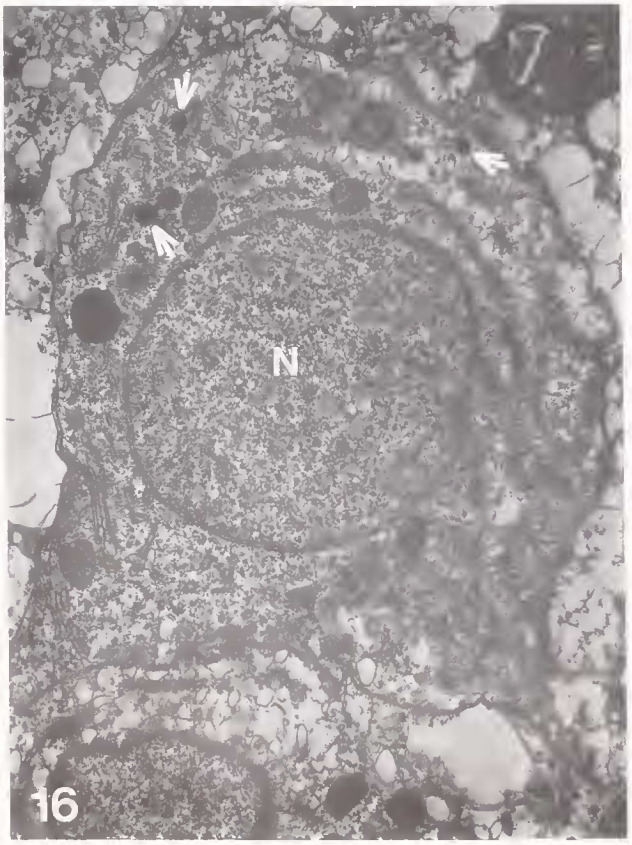
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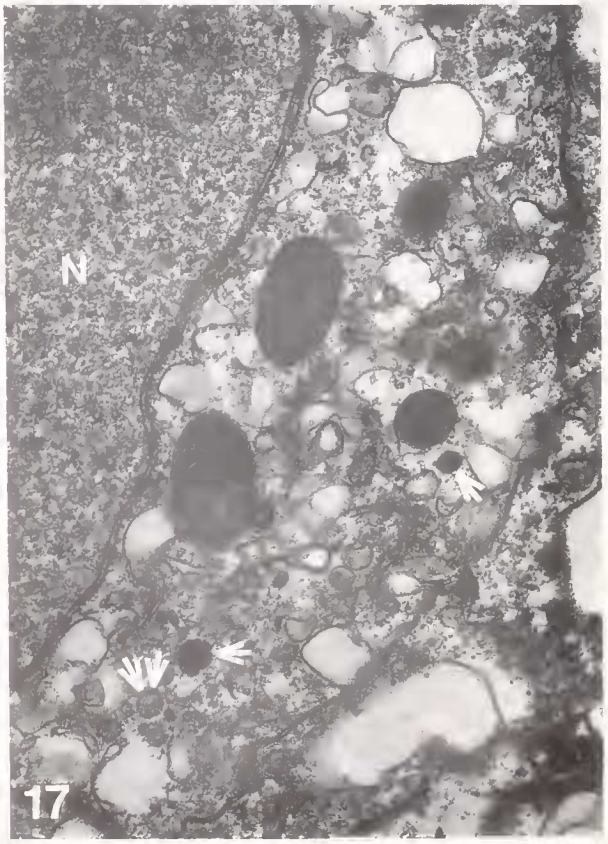
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Figure 18. Golgi region of a "neural" endodermal interstitial cell in a 48 h planula. Several mitochondria and microtubules are seen in close proximity to the Golgi. ×37,400.

Figure 19. Electron-dense droplets (arrows) in the Golgi region of a developing endodermal ganglionic cell. Such droplets are characteristic of neural differentiation. ×20,400.

mid-region of the planula. Interstitial cells destined to form ganglionic cells migrate out from all regions of the central endoderm and are evenly distributed along the planular anterior-posterior axis in the ectoderm. Since the interstitial cells and their progeny exhibit a rather precise positioning within the ectoderm, some mechanism of directed migration may be operating in the planula. The FMRFamide findings support the notion of directed migration. FMRFamide-positive endodermal interstitial cells and FMRFamide-positive ectodermal

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Figure 14. Endodermal interstitial cell in the anterior region of a 48-h planula. This undifferentiated cell contains a centrally located nucleus (N), a few segments of rough endoplasmic reticulum (arrow), a few mitochondria, and numerous free ribosomes. Although not visible in this plane of section, the interstitial cell also contains a prominent nucleolus. This interstitial cell has migrated from its site of origin in the central endoderm to the outer endoderm (E) and is in close proximity to the mesoglea (M). ×14,400.

Figure 15. Developing nematoblast in the anterior endoderm of a 48-h planula. Interstitial cells traversing the nematocyte differentiation pathway are characterized by the appearance of large amounts of rough endoplasmic reticulum (arrow) and by the formation of a nematocyst capsule (C). Such cells eventually emigrate to the ectoderm. N, nucleus. ×10,800.

Figure 16. Interstitial cell traversing the neural differentiation pathway in the anterior endoderm of a 48-h planula. Such interstitial cells committed to the ganglionic pathway form small electron-dense droplets (arrows) and dense cored vesicles (see Fig. 17), develop a Golgi (see Fig. 18), and accumulate microtubules in their cytoplasm (see Fig. 18). These cells do not develop neurites in the endoderm. N, nucleus. ×9,600.

Figure 17. Developing ganglionic cell in the endoderm of a 48-h planula. The cytoplasm of the differentiating interstitial cell becomes filled with electron-dense droplets (single arrow) and dense cored vesicles (double arrows). Similar droplets and vesicles are abundant in the cell bodies and the neurites of ectodermal ganglionic cells (see Fig. 4). N, nucleus. ×21,600.

ganglionic cells are confined to the same anterior head region of the late planula. As stated previously these positive interstitial cells probably emigrated to the ectoderm and formed the positive ganglionic cells. The fact that the FMRFamide-positive ganglionic cells are confined to a specific region in the ectoderm and not distributed at random suggests directed migration.

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